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GB Virus-C/Hepatitis G Virus Infection in Prostitutes: Possible Role of Sexual Transmission

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The modes of transmission of GB virus-C/hepatitis G virus (GBV-C/HGV) other than by blood transfusion are largely unknown. The prevalence of GBV-C/HGV viremia and the associated risk factors in 145 female prostitutes were examined. The seroprevalence of hepatitis B surface antigen (HBsAg), antibodies to hepatitis C virus (anti-HCV), and GBV-C/HGV RNA were 14%, 18%, and 11%, respectively. The demographic characteristics were similar between subjects with and without HBsAg. In contrast, those with HCV or GBV-C/HGV infection had practised longer as prostitutes and received blood transfusion more frequently. Moreover, the prevalence of GBV-C/HGV RNA and anti-HCV tended to increase in parallel with the duration of prostitution. These results suggest that like HCV, sexual transmission of GBV-C/HGV occurs and the risk increased with prolonged duration of exposure. The transmission efficiency between GBV-C/HGV and HCV appears to be similar. ***J. Med. Virol. 52:381–384, 1997.***

KEY WORDS: GB virus-C/hepatitis G virus; reverse transcription-polymerase chain reaction; 5′ untranslated region; prostitute; sexual transmission

INTRODUCTION

Despite the rapid advances in understanding of the known hepatitis viruses A to E, there still remain some cases of post-transfusion and community-acquired hepatitis with undefined etiology, suggestive of the existence of additional causative agents [Alter, 1994]. Recently, two flavi-like RNA viruses with genome sizes of 9.1 kb to 9.3 kb were identified independently in patients with chronic hepatitis and were designated GBV-C and HGV, respectively [Simons et al., 1995; Linnen et al., 1996]. Both clearly are not variants of HCV by phylogenetic analysis, and a comparison of their putative encoded polyproteins shows a high sequence identity [Zuckerman, 1996]. These facts indicate that GBV-C and HBV are independent isolates of the same virus and "GBV-C/HGV" has been widely used to name this novel virus [Zuckerman 1996; Kao et al., 1997a]. Although sensitive and specific serologic assays are not yet available, previous studies based on reverse transcription-polymerase chain reaction (RT-PCR) procedures to detect GBV-C/HGV RNA in plasma or serum samples from different populations have shown that the virus is transmissible by blood transfusion, is distributed globally, and can induce persistent viremia in humans [Simons et al. 1995; Linnen et al., 1996; Wang et al., 1996; Kao et al., 1997a]. So far, little is known about the other modes of GBV-C/HGV transmission. Sexual contact with infected partners or multiple partners has been reported to be important for transmission of hepatitis B, C, and D viruses (HBV, HCV, and HDV), but whether this route is also important for GBV-C/HGV infection remains unknown. In Taiwan, female prostitutes have been reported to be a high-risk population for HCV and HDV infection. Thus we studied the prevalence of GBV-C/HGV viremia and associated risk factors of GBV-C/HGV infection in this population.

MATERIALS AND METHODS

Subjects

One hundred and forty-five human immunodeficiency virus–negative licensed female sex workers (mean age, 35 ± 10 years; range, 20–65 years) who received mandatory routine health check-ups in the Municipal Veneral Disease Control Institute were included. They

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were asymptomatic for liver disease on enrollment, and none admitted a history of intravenous drug abuse. Serum samples taken from each subject were stored at −70°C until use.

Before blood sampling, each subject was asked to complete an interviewer-administered questionnaire addressing the duration of prostitution in years, past history of blood transfusion, surgery (including induced abortion), injection or acupuncture with nondisposable needles, tattoos or ear-piercing, sexually transmitted diseases, and intravenous drug abuse.

Serologic Testings

All serum samples were assayed for hepatitis B surface antigen (HBsAg) by a radioimmunoassay (Ausria-II, Abbott Laboratories, North Chicago, IL) and antibodies against HCV (anti-HCV) by a second-generation enzyme immunoassay (Abbott Laboratories). Serologic tests for syphilis (STS) including rapid plasma reagin (RPR), Veneral Disease Research Laboratory (VDRL), and Treponema pallidum hemagglutination (TPHA) were performed by standard procedures.

Detection and Genotyping of HCV RNA

Serum HCV RNA was assayed by reverse transcription (RT)-PCR with nested primers from the most conserved 5′ untranslated region (5′UTR) of the viral genome, and genotypes were determined by Okamoto's type-specific primers [Kao et al., 1992; 1994]. Serum samples from patients with defined types 1b, 2a, or 2b viruses infection were mixed together and used as positive controls for the PCR assay.

Detection of GBV-C/HGV RNA

Serum GBV-C/HGV RNA was detected by RT-PCR with nested primers derived from the 5′UTR [Kao et al., 1997b], which were highly conserved in both GBV-C and HGV and were distant from flaviviruses, pestiviruses, and HCV. Briefly, RNA was extracted from 100 μl of serum and converted into cDNA. For the first stage PCR, a 25 μl of reaction mixture containing 2 μl of the cDNA sample, 1× PCR buffer (10 mM tris-HCl pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin and 0.1% Triton X-100), 10 mM of each dNTP, 1 unit of Taq DNA polymerase, and 100 ng of outer sense primer 101s (5′-GGCCAAAAGGTGGTGGATGG-3′, nucleotide positions 101–120), as well as outer antisense primer 350a (5′-GGTCCACGTCGCCCTTCAAT-3′, nucleotide positions 331–350), was amplified in a thermal cycler (Perkin-Elmer Cetus, Norwalk, CT) for 30 cycles. Each cycle entailed denaturation at 95°C for 60 sec, primer annealing at 55°C for 30 sec and extension at 72°C for 60 sec. After the first amplification, 1 μl of the PCR products was reamplified for another 30 cycles with 100 ng of inner sense primer 121s (5′-GTGATGACAGGGTTGGTAGG-3′, nucleotide positions 121–140) and inner antisense primer 330a (5′-GGCAAACGACGCCCACGTAC-3′, nucleotide positions 311–330). The second round of PCR was done in the same manner as the first round. The amplified products were separated in 3% agarose gel electrophoresis and stained by ethidium bromide. The sensitivity of this assay was assessed with a titration experiment by using serial 10-fold dilutions of a serum sample known to be positive for GBV-C/HGV RNA. The viral genome became undetectable in $1:10^5$ dilution by ethidium bromide staining. The specificity was confirmed by direct sequencing of the amplified products by using fluorescence labeled primers with a 373A Sequencer (Applied Biosystems, Foster City, CA).

To avoid false-positive results in the PCR assays, serum samples from healthy persons and reagents without DNA were always used as negative controls, and instructions to prevent cross contaminations were strictly followed [Kwok and Higuchi, 1989]. Results were considered valid only when they were obtained in at least two separate runs.

TABLE I. Prevalence of Serum HBsAg and Anti-HCV in Prostitutes With and Without GBV-C/HGV Infection*

	Prostitutes with GBV-C/HGV RNA	
Viral markers	Positive	Negative
HBsAg (+)	3 (19%)	17 (13%)
Anti-HCV (+)	7 (44%)	19 (15%)
HBsAg (+), Anti-HCV (+)	0	2 (2%)
HBsAg (−), Anti-HCV (−)	6 (37%)	91 (70%)
Total	16 (100%)	129 (100%)

*HBsAg, hepatitis B surface antigen; anti-HCV, antibodies against hepatitis C virus; GBV-C/HGV, GB virus-C/hepatitis G virus.

Statistical Analysis

Data were analyzed by chi-square test with Yates' correction or Student's *t*-test, and variables that achieved statistical significance in univariate analysis were subjected to multivariate analysis (multiple logistic regression) to determine the independent significant factors. A P value < 0.05 was considered significant.

RESULTS

Of 145 licensed female prostitutes, the number with serum HBsAg, anti-HCV, and GBV-C/HGV RNA was 20 (13.7%), 26 (17.9%), and 16 (11.0%), respectively. The prevalence of HBsAg, anti-HCV, and each combination in prostitutes with and without GBV-C/HGV infection is shown in Table I. Thirty-one (21%) of them were reactive for RPR and/or VDRL tests, and only one could not be confirmed by TPHA test. The false-positive RPR test was not associated with HCV or GBV-C/HGV infection. A high frequency of surgical procedures (mostly induced abortions) (40%), tattoos (mostly on the eyebrows or eyelids for cosmetic reasons) or ear-piercing (87%), and sexual transmitted diseases (41%) were observed in this population.

The demographic characteristics with respect to mean age, mean duration of prostitution, history of blood transfusion or operation, injection or acupuncture with nondisposable needles, and tattoos or ear-

TABLE II. Demographic and Background Data Between Prostitutes With and Without GBV-C/HGV Infection*

Characteristics	Prostitutes with GBV-C/HGV RNA: Positive (n = 16)	Prostitutes with GBV-C/HGV RNA: Negative n = 129)	*P* value
Age (years)[a]	36 ± 9	35 ± 10	NS
Duration of prostitution (years)[a]	6.5 ± 3.6	4.5 ± 2.8	0.01
History of transfusion	5 (31%)	12 (9%)	0.03
History of surgery	7 (44%)	51 (40%)	NS
Nondisposable needle	4 (25%)	27 (21%)	NS
Tattoo/ear-piercing	12 (75%)	114 (88%)	NS
History of STD	8 (50%)	52 (40%)	NS
STS	4 (25%)	27 (21%)	NS

*GBV-C/HGV, GB virus-C/hepatitis G virus; STD, sexually transmitted diseases; STS, serologic tests for syphilis; NS, not statistically significant.
[a]Mean ± standard deviation.

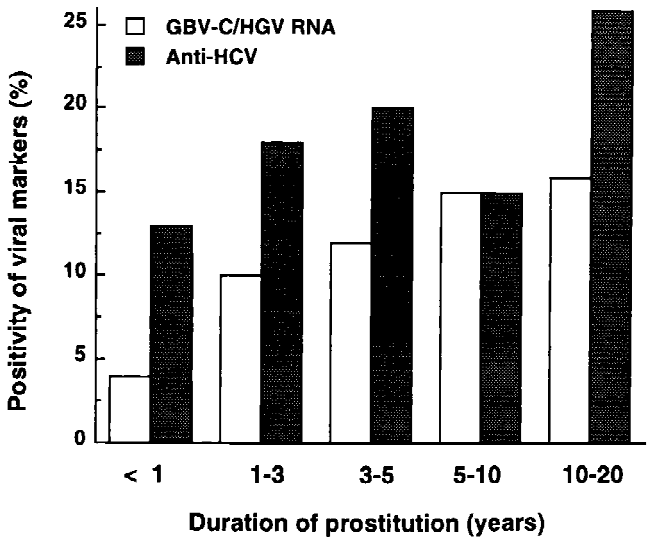

Fig. 1. Prevalence of serum GBV-C/HGV RNA and anti-HCV in female sex workers by their duration of prostitution. The positive rates of GBV-C/HGV RNA and anti-HCV are shown by open bars and shaded bars, respectively. The calculated slopes for the increment of GBV-C/HGV RNA and anti-HCV were 1.45 and 1.15, respectively.

piercing between subjects with and without HBsAg carriage were comparable. Of 26 anti-HCV-positive patients, 7 (27%) were coinfected with GBV-C/HGV, and 20 (77%) were positive for serum HCV RNA. Genotyping of HCV showed type 1b in 8 (40%), type 2a in 5 (25%), type 2b in 6 (38%), and mixed infections in 1 (5%). Although no statistical significance was reached, anti-HCV-positive prostitutes appeared to have a longer duration of prostitution (5.4 ± 3.4 years vs. 4.6 ± 3.8 years) and a higher frequency of past transfusion (23% vs. 9%) and surgery (58% vs. 36%) than the seronegatives. The distribution of other risk factors was similar between the two groups (data not shown). In GBV-C/HGV infection, the mean duration of prostitution in those with viremia was significantly longer than that of those without viremia (6.5 years vs. 4.5 years, $P = 0.01$) and more of the GBV-C/HGV RNA-positives had blood transfusions before (31% vs. 9%, $P = 0.03$) on univariate analysis (Table II). By multivariate analysis, mean duration of prostitution and blood transfusion were positively associated with GBV-C/HGV infection. Moreover, the prevalence of GBV-C/HGV RNA and anti-HCV tended to increase in parallel with the duration of prostitution, and the calculated slope of the GBV-C/HGV curve was slightly steeper than that of the anti-HCV curve (Fig. 1).

Because of a high percentage of surgical procedures, tattoos or ear-piercing, and sexually transmitted diseases in these subjects, it was difficult to show an association between these factors and HCV or GBV-C/HGV infection.

DISCUSSION

With the discovery of viral genome and subsequent development of molecular diagnostic assays, the epidemiology and clinical significance of GBV-C/HGV infection have been partially understood [Simons et al., 1995; Linnen et al., 1996; Wang et al., 1996; Kao et al., 1997a]. Several studies have indicated that GBV-C/HGV is a parenterally transmitted virus, and its infection seems not to cause significant hepatitis as the case with hepatitis viruses A–E [Simons et al., 1995; Linnen et al., 1996; Wang et al., 1996; Kao et al., 1996; 1997a]. In addition, GBV-C/HGV co-infection is found frequently in hepatitis B and C carriers, suggesting these viruses share similar modes of transmission [Linnen et al., 1996; Kao et al., 1997a]. In Taiwan, we have recently shown that the prevalence of GBV-C/HGV viremia in healthy adults, patients with chronic non-B, non-C hepatitis, HBV, and HCV carriers were 1%, 10%, 3.2%, and 10%, respectively, and its co-infection does not aggravate the clinical course of chronic hepatitis B or C [Kao et al., 1997a].

Transmission of GBV-C/HGV by blood transfusion or by parenteral exposure is well documented, but little is known about the possibilities of non-parenteral routes of transmission. Feucht et al. [1996] reported that three infants (33.3%) of nine high-risk GBV-C/HGV-positive mothers had perinatal transmission of GBV-C/HGV. In contrast, our data showed that perinatal GBV-C/HGV transmission perhaps does not occur frequently in the low-risk population [Lin et al., 1996]; however, the number of subjects studied was limited, and further observations on more cases of GBV-C/HGV viremic mothers and their children are warranted. Meantime, although sexual contact with infected partners or multiple partners has been shown to be one of the important routes for contracting HBV, HCV, and HDV infections, whether it also works in GBV-C/HGV infection is virtually unknown.

The prevalence of serum HBsAg, anti-HCV, and GBV-C/HGV RNA was investigated in female sex workers, who are known to be susceptible to many viral infection by sexual contact, and the associated risk factors were analyzed. The results showed that the positive rate of serum HBsAg in this setting was 14%, simi-

lar to that in age-matched general population [Kao et al., 1997a]. This is not surprising because most HBsAg carriers in Taiwan acquired HBV infection in early childhood. In contrast, the prevalence of anti-HCV (18%) and GBV-C/HGV RNA (11%) in these prostitutes were 10- to 20-fold higher than that of the general population [Kao et al., 1997a]. Additionally, the true prevalence of GBV-C/HGV infection may actually be underestimated, because we relied solely on PCR assay to detect viremia in the present study. Further development of specific serologic assays for markers of GBV-C/HGV infection are needed to unravel the true infection rate of this virus.

Co-infection of GBV-C/HGV has been observed with a frequency of 10%–19% in patients with chronic hepatitis C [Linnen et al., 1996; Kao et al., 1997a]. Our findings that 44% of the GBV-C/HGV-infected prostitutes were also positive for anti-HCV imply GBV-C/HGV and HCV indeed share common modes of transmission (Table I). Studies to explore the clinical implications and interactions between HCV and GBV-C/HGV—all flavi-like viruses—in the coinfected patients are ongoing in our laboratories.

Several studies have shown that the risk of HCV transmission through sexual contact is small but cumulative [Kao et al., 1992; Wu et al., 1993]. In the present study, the fact that anti-HCV-positive prostitutes had a longer period of prostitution than the seronegatives is in keeping with our previous observations that spouses of HCV-infected patients with longer exposure duration are at increased risk of acquiring HCV infection [Kao et al., 1992]. Interestingly, when GBV-C/HGV-infected prostitutes were compared to those not infected, the association between longer duration of prostitution and viral infection became even more evident (Table II). These data suggested that both HCV and GBV-C/HGV can spread through sexual transmission, and prostitutes may serve as a reservoir for these viruses. Further analysis showed that prevalence of GBV-C/HGV RNA and anti-HCV increased in parallel with the duration of prostitution, and the calculated slopes of GBV-C/HGV curve and anti-HCV curve were similar, suggesting the transmission efficiencies of HCV and GBV-C/HGV through sexual contacts are comparable. However, the efficiency of GBV-C/HGV transmission was likely underestimated because the infection was documented by the presence of viremia only, and not all GBV-C/HGV infected subjects become chronic carriers of the virus [Wang et al., 1996].

Most (77%) of the anti-HCV-positive prostitutes were viremic, and HCV type 1b was found in only 40%, as compared to 70–80% of patients with chronic hepatitic C in our previous reports. This discrepancy may be attributed to the younger and asymptomatic subjects included in the present study, or, less likely, selective transmission of certain genotypes of HCV through sexual contact. Another possibility is that the prevailing HCV genotype is undergoing changes currently in Taiwan as has been documented in France and Italy [Nousbaum et al., 1995].

In summary, as with HCV, sexual transmission of GBV-C/HGV may exist in prostitutes and the risk of infection may increase with prolonged exposure to multiple sexual partners. The efficiency of GBV-C/HGV transmission seems comparable to that of HCV. Female prostitutes are not only a high-risk population for both HCV and GBV-C/HGV infection but may also be a reservoir of these viruses.

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